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AUTHOR(S):

NAGATA, Yoshinosuke; KITAO, Koichiro; TACHI, Isamu

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Polarographic Studies on the Heartwood Flavonoids. II.

Copper Chelate Compounds with Dihydroquercetin and Quercetin.

Division of Wood Chemistry, Section 1

Yoshinosuke NAGATA*, Koichiro KITAO and Isamu TACHI

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Introduction

In the previous paper¹⁾, the polarographic investigations on dihydroquercetin and quercetin have been carried out. It has been clarified that the electron numbers involved in the reduction reaction were two, both compounds were reduced to alcohol, and the separatory determination of both was possible in the acetate-buffered solution.

It has been found polarographically by W. E. Detty, *et al*²⁾ that various flavonoids with OH group at 3-position had the possibility of chelation with metallic ions, especially copper ion to form a stable chelate in the neutral buffered solutions. And it has been further proved spectrophotometrically that dihydroquercetin and quercetin could be used as the chelating agent for zirconium and aluminium^{3,4)}. The present authors, in this paper, illustrate the polarographic behavior of copper chelate with dihydroquercetin and quercetin in the several kinds of buffered solutions, in which one mol. copper combines with 2 mols. flavonoids. In the sulfuric acid solution, the soluble chelate gives two-step reduction wave at more negative potential than the wave of copper, the limiting current of which indicates the character of the kinetic current due to the dissociation reaction of the chelate to cupric ion and chelating agent. The ratio of copper to quercetin for the chelate formation is one to one in the sulfuric acid solution.

Experimental

Materials Dihydroquercetin and quercetin are prepared and purified as mentioned in the previous paper. Buffered solutions used are McIlvain's citrate, Kolthoff's phosphate, Sørensen's borate, and Michaelis' acetate buffered solutions.

Method Yanagimoto Polarograph and Galvarecorder are used, and Yanagimoto Polarograph PB-II is used in the case of A.C. polarography.

The half-wave potentials are determined against the normal calomel electrode

* Dept. of Agr. Chem., Faculty of Agr., Kyoto University.

without correcting the iR drop.

The flow-rate of mercury out of the capillary used for the dropping mercury electrode, m , is 1.16 mg/sec and drop time, t , 4.28 sec; the characteristic of the electrode, $m^{2/3}t^{1/6}$, is 1.75 in 0.2 N KCl solution at -1.5 v. vs. N.C.E.

The electrolytic solutions used in this work contain 10 % and 30 % ethanol in the case of dihydroquercetin and quercetin, respectively, because of less solubility of these compounds in water. The supporting electrolytes, such as KCl and KNO_3 , are not added in order to avoid the complexity of the polarographic behavior due to the complex formation of copper with the supporting electrolyte anions.

The pH values of the solutions are measured with a Horiba's HRL pH meter Model H. Dissolved oxygen is removed from all solution with pure nitrogen gas bubbling through the solution. All experiments are carried out at $30 \pm 0.5^\circ C$.

Results and Discussion

I. Chelates of Dihydroquercetin

Comparing the polarographic behavior of Cu^{++} with that of Cu^{++} +dihydroquercetin, as shown in Table 1, the Cu wave is considerably influenced by the addition of dihydroquercetin in certain pH range. Columns 2 and 3 in Table 1 represent the half-wave potential and the limiting current of the copper reduction at absence of dihydroquercetin (A), respectively. Cupric ion does not give the reduction wave at pH 10.9. The solution of $5 \times 10^{-4} M$ Cu^{++} and dihydroquercetin

Table 1. Relationship between the Half-Wave Potential, the Limiting Current and pH. (A): $5 \times 10^{-4} M$ Cu^{++} , (B): (A)+ $5 \times 10^{-4} M$ Dihydroquercetin.

pH	(A)		Coloration Ppt.	(B)				Chelat- ing Ratio
	$E_{\frac{1}{2}}$	i		$E_{\frac{1}{2}}$		i		
				(1)	(2)	(1)	(2)	
2.30 ^a	-0.00v.	1.92 μ A	Colorless	-0.00v.	—	1.88 μ A	—	1 : 2
4.80 ^a	-0.12	1.94	“	-0.09	—	1.84	—	
8.05 ^b	-0.18	0.60	“	-0.20	-0.40v.	0.24	0.06 μ A	
10.9 ^c	—	—	greenish yellow	-0.15	-0.45	0.14	0.70	
4.8 ^d	-0.05	2.20	coloreless	-0.09	—	2.18	—	1 : 2
6.0 ^d	-0.07	2.06	pale greenish yellow. ppt.	-0.05	-0.24	0.92	0.26	
0.5M AcONa	-0.01	1.42	“	-0.05	-0.24	0.68	0.16	
0.1N H ₂ SO ₄	+0.03	2.36	colorless	+0.04	-0.58	1.96	0.40	

a: McIlvain's buffer, b: Kolthoff's, buffer, c: Sørensen's buffer, d: Michaelis' buffer.

(B) shows specific coloration and precipitation (column 4). It indicates no chelate formation that the difference in the limiting currents between (A) and (B) solutions is not seen at pH 2.30, 4.80 and 4.8 (acetate buffer) (columns 5 to 8). On the other hand, the B solutions at pH 8.05 and 6.0 (acetate buffer), and in 0.5M sodium acetate give two reduction waves, the first of which corresponds to the reduction of Cu^{++} since its half-wave potential agrees with that of A. The second wave seems to be due to the reduction of a chelate formed. However, the limiting current of the second wave is much smaller than the expected value, which is slightly smaller than the first owing to decrease in the diffusion coefficient resulting from the chelate formation. The precipitate is observed in these B solutions. It is, accordingly, considered that the limiting current of the second wave corresponds to the soluble part of chelate in the solution. This assumption is probable from the fact that the second wave increasing with increasing the content of alcohol in the solution, though the first wave height decreases which is a normal effect (Table 2).

Table 2. Influence of the Content of Ethanol on the Wave Height of $5 \times 10^{-4}\text{M}$ Cu^{++} and Dihydroquercetin in Acetate Buffer of pH 6.0.

Conc. of Ethanol	i_1	i_2
10 %	0.84 μA	0.18 μA
20	0.80	0.20
30	0.66	0.22
40	0.68	0.26
50	0.54	0.20

The diffusion current of the first wave is equivalent to a half of that of (A) by addition of equimolar concentration of dihydroquercetin. Fig. 1 indicates the titration* of cupric ion with dihydroquercetin (curve 1) and the reverse one (curves 2 and 3) in the acetate buffered solution of pH 6.0. The same results are obtained in the other

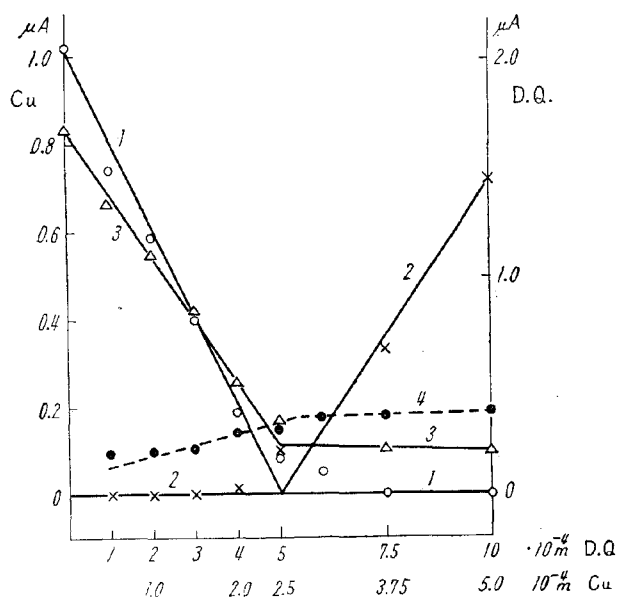


Fig. 1. Amperometric titration in acetate buffered solution of pH 6.0. (1) Cu^{++} wave and (4) the chelate wave in the titration of $2.5 \times 10^{-4}\text{M}$ Cu^{++} with dihydroquercetin. (2) Cu^{++} wave and (3) the dihydroquercetin wave in the titration of $5 \times 10^{-4}\text{M}$ dihydroquercetin with Cu^{++} .

* For example, each point on the curve 1 in Fig. 1 is measured separately in the individual electrolytic solution which contains $2.5 \times 10^{-4}\text{M}$ Cu^{++} and certain concentration of dihydroquercetin. Strictly speaking, therefore, this method is not the amperometric titration.

pH solutions which are regarded to form a chelate. Consequently, it is concluded from Fig. 1 and Table 1 that the chelating ratio of copper and dihydroquercetin is one to two.

However, as shown in Fig. 1 (curve 1), even when dihydroquercetin is added in copper solution by equivalent amount, a small copper wave remains. Moreover, in the case of reverse addition, the dihydroquercetin wave does not disappear completely (curve 3). It would give an explanation to this fact that the soluble chelate formed is partly dissociated in cupric ion and dihydroquercetin.

Table 3. Effect of the Mercury Reservoir Height (h) on the Limiting Current of $5 \times 10^{-4}M$ Cu^{++} and Dihydroquercetin in Acetate Buffer at pH 6.0.

h	\sqrt{h}	i_1	i_2
80 cm	8.96	1.28 μA	0.22 μA
70	8.37	1.18	0.20
60	7.70	1.12	0.22
50	7.07	1.04	0.20
40	6.32	0.96	0.18
30	5.48	0.84	0.18
Slope at h=50 cm		0.118	

The second wave height which is represented by the dotted line in Fig. 1 (curve 4), keeps nearly a constant value, which seems to depend on the solubility of the chelate. Table 3 gives the relationship between the limiting current and the mercury reservoir height. It is seen from the slope of the limiting current against the square root of the Hg reservoir height that the first wave is controlled nearly by diffusion process⁵⁾. Nevertheless, the second limiting current due to chelate is almost unaffected by the variation in the mercury pressure. It is, therefore,

considered that the second wave contains the character of so-called kinetic current⁶⁾, which is based on the chelate forming reaction in the equilibrium $Cu^{++} + 2$ dihydroquercetin \rightleftharpoons Cu(II)-chelate.

The investigations in sulfuric acid solution are mentioned later.

II. Chelates of Quercetin

All experiments are conducted in 30 % alcoholic solution since quercetin is not dissolved in 10% alcoholic solution. The reduction wave of copper in the absence of quercetin (A') in the alkaline solution is not observed because of basicity and the effect of alcohol. Therefore, the polarographic studies in such solutions are excluded.

Table 4 shows the influence of pH on the formation of chelate in the same manner as in Table 1. A single reduction wave is observed in the acetate buffered solution of $5 \times 10^{-4}M$ Cu^{++} and quercetin (B') and its wave height is equal to a half of that obtained in A' solution. This wave is due to the reduction of Cu^{++} from a good agreement of both half-wave potentials. No reduction wave is obtained for the chelate, which probably precipitates in the solution.

Table 4. Relationship between the Half-wave Potential, the Limiting Current and pH. (A'): $5 \times 10^{-4} \text{M Cu}^{++}$, (B'): (A') + $5 \times 10^{-4} \text{M Quercetin}$.

pH	(A')		Coloration Ppt.	(B')				Chelat- ing Ratio
	$E_{\frac{1}{2}}$	i		$E_{\frac{1}{2}}$		i		
				(1)	(2)	(1)	(2)	
2.30 ^a	-0.17v.	1.52 μ A	pale yellow	-0.19v.	—	1.46 μ A	—	1 : 2
4.80 ^a	-0.16	1.44	// ppt.	-0.17	-0.55v.	1.04	0.36 μ A	
8.05 ^b	—	—	brown	—	-0.36	—	0.24	
10.9 ^c	—	—	brownish yellow	—	-0.46	—	0.34	
4.8 ^d	-0.19	1.88	yellow ppt.	-0.15	—	1.00	—	
6.0 ^d	-0.10	1.28	//	-0.05	—	0.46	—	
0.5M AcONa	-0.05	0.24	//	—	—	—	—	
0.1N H ₂ SO ₄	+0.05	1.96	pale yellow	+0.07, -0.15, -0.70	0.42, 1.08, 0.54		1 : 1	

a: McIlvain's buffer, b: Kolthoff's buffer, c: Sørensen's buffer, d: Michaelis' buffer.

The ratio of chelation, as given in Fig. 2, is one to two as well as in the case of dihydroquercetin, while the chelate formation is complete unlike the dihydroquercetin chelate.

The polarographic determination of the stability constant is performed from the relationship between the half-wave potential shift of the chelate reduction wave and the concentration of the chelating agent. Unfortunately, since the chelating agent with high concentration is not dissolved in the alcoholic solution, the stability constant of the chelate cannot be measured polarographically in this work.

III. Chelates in Sulfuric Acid Solution

Two step reduction wave is obtained in $5 \times 10^{-4} \text{M Cu}^{++}$ and dihydroquercetin solution containing 0.1N sulfuric acid. The first wave is accompanied by the

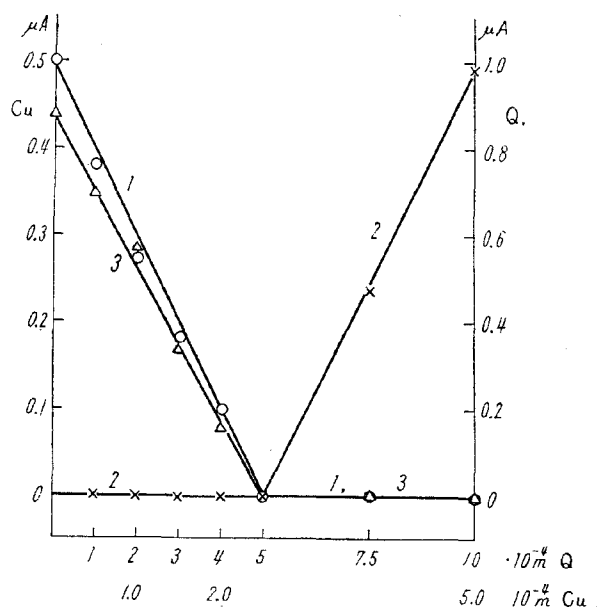


Fig. 2. Amperometric titration in acetate buffered solution of pH 6.0. (1) Cu^{++} wave in the titration of $2.5 \times 10^{-4} \text{M Cu}^{++}$ with dihydroquercetin. (2) Cu^{++} wave and (3) quercetin wave in the titration of $5 \times 10^{-4} \text{M Quercetin}$ with Cu^{++} .

maximum-like phenomenon, which is suppressed by the addition of gelatin solution. However, the resulting limiting current is equal to that without gelatin and the limiting current of the second wave decreases. This fact indicates that the apparent maximum phenomenon comes to the minimum one.

The half-wave potential of the first wave, as shown in Table 1, agrees with

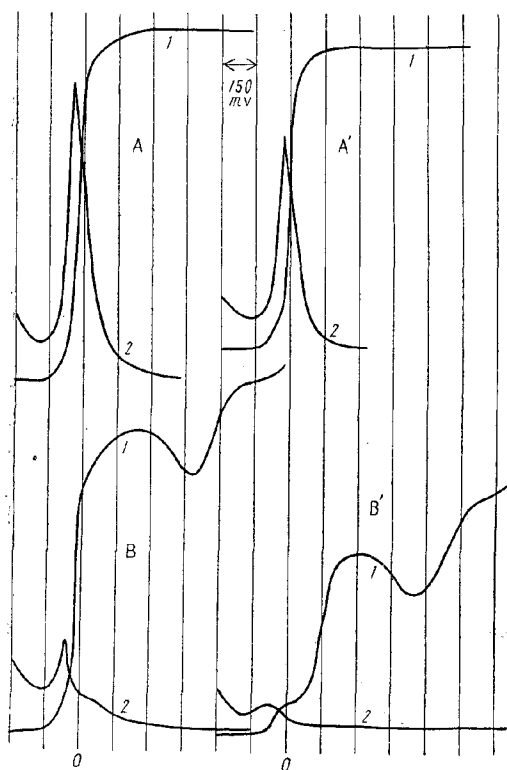


Fig. 3. D.C. and A.C. polarograms in 0.1N sulfuric acid solution. (1) D.C. polarograms, and (2) A.C. polarograms. $5 \times 10^{-4}M$ Cu^{++} (A) in 10% (A') 30% ethanol. (B): (A) + $5 \times 10^{-4}M$ dihydroquercetin, (B'): (A') + $5 \times 10^{-4}M$ quercetin.

that of copper in the solution not containing dihydroquercetin. Fig. 3 gives the relationship between the D.C. polarograms and A.C. polarograms of A, A', B and B' in 0.1N sulfuric acid. Curves 1 and 2 represent D.C. and A.C. polarograms, respectively. In A.C. polarography, the peak current in B solution is considerably smaller than in A solution. In general, the A.C. polarographic wave appears in the reversible system and the peak current of the A.C. wave is proportional to the reversibility of the corresponding system under a definite condition. Since the reduction of the chelate is nearly irreversible, the A.C. wave corresponding to the first wave in B solution seems to be not due to the chelate but to the copper present without chelating. It is, therefore, adequate to consider that both waves of copper and chelate in D. C. polarogram are overlapping each other in the first wave. This behavior makes the determination of chelating ratio impossible and other polaro-

graphic explanation very complicated and difficult.

On the other hand, three step reduction wave is seen in the case of quercetin. The first wave with a small limiting current is due to the reduction of copper, which is not combined with quercetin as the chelate, from a view-point of D.C. polarography (coincidence of the half-wave potentials) and A.C. polarography (agreement of the summit potentials) (Fig. 3).

The minimum phenomenon appears at the initial part of the third wave in the same way as with dihydroquercetin. It can be said also from this similarity that the waves of the free cupric ion and chelate overlap each other in the first waves in B solution. It is, therefore, concluded from the results of A.C. polaro-

graphy and the wave analysis that the first and second wave of B and the second and third waves of B' is due to the reduction of the chelate formed.

The variation in the concentration of sulfuric acid influences on each limiting current of three reduction waves as shown in Fig. 4. The first waves height decreases abruptly with increasing the concentration of sulfuric acid and keeps constant value in the range over 0.5N. The second wave decreases and the third increases gradually with increasing the sulfuric acid concentration. The decrease in the first wave height means that the formation of chelate is stabilized by the presence of sulfuric acid. The variations in the second and third wave heights correspond to the stability of any two types of chelate.

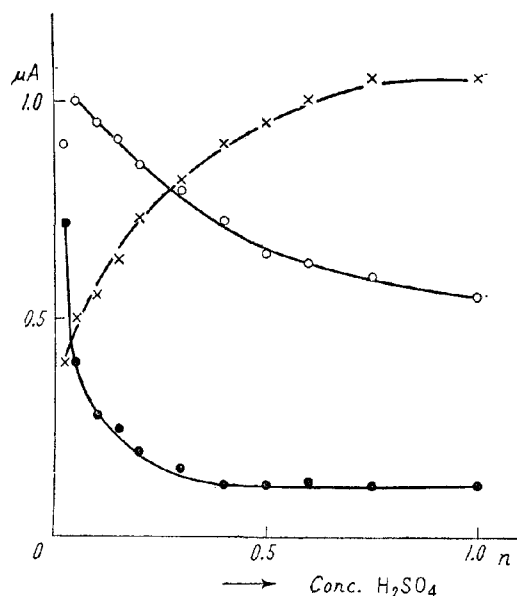
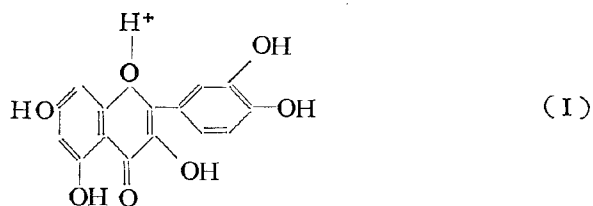


Fig. 4. Influence of the H_2SO_4 concentration on the wave heights of $5 \times 10^{-4}M$ Cu^{++} and quercetin solution containing 30 % ethanol at $30^\circ C$.
 —●— : first wave, —○— : second wave, —×— : third wave.

The following assumptions are presented for the reduction mechanism of the chelate wave. At first, the cupric chelate is reduced in stepwise to metallic copper through the cuprous chelate. To ensure this consideration, two waves must be equal. Nevertheless, both waves vary with the concentration of sulfuric acid, which is contradictory to the first assumption. Secondly, flavonoid has a possibility to form a cationic structure (I) in acidic solution, which is more reducible than the molecule; i.e., the cation wave must increase with increasing



of acidity. However, it is consistent that the second wave due to cation decreases with increasing the sulfuric acid concentration. In short, the variations in the second and third waves with the concentration of sulfuric acid can be explained favorably.

The ratio of copper and quercetin for chelate formation is equal to one to one from the results of the same experiment as in the buffered solution mentioned above. Accordingly, it is necessary to settle the position to form the chelate ring. The one is CO at 4-position and OH at 3-position, and the other is perhaps OH

Table 5. Effect of the Mercury Reservoir Height on the Limiting Current of $5 \times 10^{-4}M$ Cu^{++} +quercetin in $1N H_2SO_4$ solution.

h	\sqrt{h}	i_1	i_2	i_3
80 cm	8.94	0.12 μA	0.70 μA	1.42 μA
70	8.37	0.10	0.66	1.32
60	7.70	0.08	0.64	1.18
50 q	7.07	0.08	0.60	1.02
40	6.32	0.08	0.58	0.86
30	5.48	0.08	0.50	0.72
Slope at $h=50$ cm			0.126	0.198

at 3' and 4'-positions.

The first wave does not disappear completely even by the excess addition of quercetin. This suggests that the cupric ion is present without forming a chelate in the solution. Table 5 indicates the relationship between the limiting current and Hg reservoir height. The observed slopes of the limiting current of the second and third waves against the square root of the Hg pressure agree well with

the theoretical value, which is 0.141 at $h=50$ cm, in the case of the diffusion current. Hence, the limiting currents of the second and third waves are controlled by the diffusion. On the other hand, the slope of the first wave is negligible small. This represents that the limiting current contains the character of kinetic current, which seems to be based on the dissociation of chelate. Therefore, it is seen that its stability constant is considerably small. However, the stability of the chelate is greater than that of dihydroquercetin chelate, since the half-wave potential of the dihydroquercetin chelate is more positive than that of quercetin chelate.

This is imagined also from the structure of the chelating agent; i.e., the difference of the structure effects on the stability of the chelate. The planar structure of the chelate is stabilized by the resonance effect resulting from the existence of the double bond in 2,3-position; that is, the quercetin chelate is more stable than the chelate of dihydroquercetin which has not a double bond at 2,3-position.

Summary

Dihydroquercetin and quercetin form chelates with copper under certain conditions. The combination ratio of copper and both flavonoids is one to two in the buffered solution and one to one in sulfuric acid solution. The reduction wave of chelate in the acetate buffered solution of pH 6.0 has a character of kinetic current due to the chelate formation equilibrium. The quercetin chelate in sulfuric acid solution shows two step reduction wave and another one due to copper reduction is given at more positive potential, which has a character of kinetic current based on the dissociation of chelate formed. It is considered from the difference of the half-wave potentials that the stability of quercetin chelate is greater qualitatively than that of

dihydroquercetin chelate. And this is explained by the structural effect of chelate.

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